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TECHNICAL MANUSCRIPT 293

ROLE OF ETHYLENE IN LEAF ABSCSSION

Frederick B. Abeles  
Bernard Rubinstein

MAY 1966

UNITED STATES ARMY  
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**U.S. ARMY BIOLOGICAL CENTER  
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**TECHNICAL MANUSCRIPT 293**

**ROLE OF ETHYLENE IN LEAF ABSCISSION**

**Frederick B. Abeles**

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**Crops Department  
BIOLOGICAL SCIENCES LABORATORY**

**Project 1L013001A91A**

**May 1966**

FOREWORD

Mr. Rubinstein is associated with the Department of Botany, University of California, Berkeley, California.

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ABSTRACT

Abscission zone explants of Gossypium hirsutum L., Cassia fistula L., and Coleus blumei Benth. were used to investigate correlations between endogenous rates of ethylene evolution and time of abscission. Additions of 0.1 nanoliter per milliliter ethylene to the explants markedly accelerated abscission; continuous aeration of the explants, to prevent accumulation of small amounts of endogenously produced ethylene, inhibited abscission compared with that of sealed controls. Substances that stimulated abscission simultaneously accelerated ethylene evolution on all three species and at any position of application.

The positional effects of auxin are explained as being due to differences in transport in the explant. Thus, distally applied auxin inhibits abscission regardless of the accelerated rate of ethylene evolution by being rapidly transporied to the abscission zone. Auxin applied proximally stimulates abscission because it is unable to move as rapidly to the abscission zone and the ethylene effect becomes dominant.

Ethylene was found to be most effective at longer exposures and on aged tissues, and it is concluded that abscission rates are not determined basically by an auxin-ethylene balance but by an increase in sensitivity of the tissue to the ethylene that is already being produced.

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## I. INTRODUCTION

Any attempt to explain the various processes involved in leaf abscission must take into account the wide range of substances that profoundly affect its course. Auxins,<sup>1</sup> gibberellins,<sup>2</sup> ethylene,<sup>3</sup> amino acids,<sup>4-6</sup> defoliants of widely divergent chemical structures,<sup>6-8</sup> dormin (abscisin II),<sup>9-11</sup> and various plant products<sup>12-14</sup> have all been reported to hasten abscission rates.

The relationship of ethylene production to abscission may hold the key to the stimulatory activity of the above substances. Ethylene is known to be evolved from leaves,<sup>15,16</sup> and applications of defoliants<sup>16</sup> and indoleacetic acid (IAA)<sup>17</sup> increase its rate of evolution from leaves of intact plants. Results of our investigations on bean explants have indicated that substances that accelerate abscission of these explants also increase the rate of ethylene production prior to separation. It was also found that aeration of the explants somewhat retarded the activity of these promotive compounds.<sup>16,18</sup> It thus began to appear that the common bond among all substances that stimulate abscission is their ability to promote ethylene evolution.

In an attempt to investigate this notion further and to extend our findings, techniques similar to those previously reported have been employed on abscission zone explants from plants of diverse taxa. The results are compared with those reported in the earlier literature and an attempt was made to integrate conclusions from earlier reports into a more unified theory concerning the mechanisms of leaf abscission.

## II. MATERIALS AND METHODS

Four *Gossypium hirsutum* L. var. Acala 4-42 (cotton) seedlings were grown in soil-filled 10-cm pots at 26 ± 2°C under 1200 ft-c of fluorescent light and a 12-hour photoperiod. Explants were isolated after 3 weeks so as to include 10 mm of the hypocotyl and 3-mm stumps of the cotyledonary petioles. This abscission test with cotton cotyledonary node is a modification of that described by Carns et al.<sup>19</sup>

*Cassia fistula* L. was grown in soil-filled galvanized cans (45 cm high and 45 cm in diameter) in the greenhouse. Mature pinnately compound leaves were harvested from plants 6 months or more of age. Explants consisted of the rachis and pulvini of leaflets three through seven, counting as number one the leaflet nearest the bottom. Each explant measured 10 mm, with 2 mm of rachis tissue above the junction of the pulvini and 8 mm below.

A clone of coleus (Coleus blumei Benth.) was grown in the greenhouse in 10-cm pots containing soil. During the winter months they received 4 hours' additional illumination from 150-watt incandescent bulbs spaced 4 meters apart and 2 meters above the bench. Abscission zone explants were harvested from plants containing 6 to 8 nodes. Node number one was the uppermost node bearing leaves with petioles longer than 5 mm. Each explant from nodes 3, 4, and 5 consisted of 3 mm of stem tissue above the node, 10 mm below the node, and two petiole stumps 5 mm long.

Six ml of 1% agar were poured into  $43 \pm 2$ -ml gas collection bottles (5 cm in diameter and 2.5 cm high) and 10 explants of cassia or cotton or 5 coleus explants were inserted into the agar so that 3 mm of the explant were submerged. Compounds were applied at three different points to explants: bottom and top of stem (proximal applications) and petiole or pulvinal stump (distal application). Bottom applications were made by incorporating the chemicals into the agar medium into which explants were inserted. The other applications were made by incorporating chemicals into 1% agar and placing drops either on stem tissue above the node (top) or on the petiole or pulvinal stumps.

The bottles were sealed with 25-mm-diameter vaccine caps when samples of gas were to be collected or they were covered with four layers of moistened cheesecloth when the contents were to be aerated. The explants were incubated at  $25 \pm 1$  C under 150 ft-c continuous light throughout the experiment.

Abscission was measured by counting those stumps that separated when a pressure up to 10 grams was applied. The measurement of ethylene by gas chromatography has been described earlier<sup>18</sup> and is expressed as nanoliters per milliliter (nl/ml). Measurement of carbon dioxide and oxygen was based on methods outlined by Burchfield and Storrs.<sup>20</sup>

The following abbreviations are used: indoleacetic acid (IAA), naphthaleneacetic acid (NAA), gibberellic acid K salt (GA), 3,6-endoxo-hexahydrophthalic acid (endothal), phenoxyacetic acid (PAA), 2-chlorophenoxyacetic acid (2-C1), 4-chlorophenoxyacetic acid (4-C1), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,5-dichlorophenoxyacetic acid (2,5-D), 2,6-dichlorophenoxyacetic acid (2,6-D), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), and 2,4,5-trichlorophenoxyisobutyric acid (2,4,5-TISB).

### III. RESULTS

The kinetics of ethylene evolution were first determined for each type of explant employed, to serve as a guide for experimentally induced modifications to follow. As shown in Table 1, a similar pattern of ethylene production occurs in the three species studied. Ethylene is produced most rapidly during the first 6 hours after cutting the explants; the rate decreases, then it becomes fairly steady. This initial burst has been observed in other tissues and may represent a wounding response or a release of internally accumulated gas.

TABLE 1. ENDOGENOUS ETHYLENE EVOLUTION FROM ABSCISSION ZONE EXPLANTS

Plant	Explants per Bottle	<u>nl Ethylene/ml Gas Phase Accumulated<sup>a/</sup></u>				% Abscission at 72 Hours
		0-6 Hours	6-24	24-48	48-72	
Cassia	10	0.3	0.02	0.04	0.06	15
Coleus <sup>b/</sup>	5	0.8	0.6	0.2	0.1	95
Gossypium	10	0.4	0.3	0.2	0.1	20

a. Accumulated ethylene was measured after time intervals indicated.  
Bottles were vented and resealed after each measurement.

b. Explants from node 4.

Slight manipulations with the ethylene level surrounding the explants can have striking effects on abscission rates (Table 2). Ethylene levels were elevated by injections of the gas after sealing the bottles or lowered by covering the bottles with four layers of moist cheesecloth. Quite clearly, the rate of explant abscission in the cloth-covered bottles (aerated) is reduced compared with that of the controls, which were vented only at the specified times (sealed). Injections of ethylene, even at 0.1 nl/ml, markedly stimulated the abscission rate, and this stimulation appeared to be proportional to the concentration of gas added. Results of experiments with explants from the fifth node of coleus are presented here, but similar data were obtained with explants from the third and fourth nodes.

TABLE 2. EFFECTS OF AERATION AND ETHYLENE ADDITIONS  
ON ABSCISSION RATES OF EXPLANTS

Plant	Treatment <sup>a/</sup>	Ethylene (nl/m <sup>3</sup> ) Accumulated from 6-24 hr in Sealed Bottles	% Abscission		
			1 day	2 days	3 days
Cassia	Aerated	0.05	0	0	5
	Sealed		0	0	15
	0.1 nl C <sub>2</sub> H <sub>4</sub> /ml		0	5	100
	0.25 nl C <sub>2</sub> H <sub>4</sub> /ml		0	50	100
Coleus (node 5)	Aerated	0.7	0	55	95
	Sealed		0	80	95
	0.1 nl C <sub>2</sub> H <sub>4</sub> /ml		45	100	100
	0.5 nl C <sub>2</sub> H <sub>4</sub> /ml		75	100	100
Gossypium	Aerated	0.1	0	0	30
	Sealed		0	15	55
	0.1 nl C <sub>2</sub> H <sub>4</sub> /ml		30	100	100
	0.5 nl C <sub>2</sub> H <sub>4</sub> /ml		70	100	100

a. Bottles were vented 6 hours after they were originally sealed. The bottles were then examined, vented, resealed, and ethylene was again injected after 6 hours, 24 hours, and 48 hours.

Another parameter investigated was the variation in sensitivity to ethylene as a function of the age of the explants. Freshly cut, 24- and 48-hour-old cotton explants were exposed to atmospheres containing 0.25 nl ethylene per ml gas phase. The aged explants were kept the specified lengths of time prior to the experiment in cloth-covered bottles. The data in Figure 1 show that up to seven hours of ethylene exposure caused minimal differences in abscission. However, by 21 hours it was apparent that explants aged for 24 hours had abscised most rapidly. Older explants responded less rapidly to the ethylene, and explants that were freshly cut at the beginning of the experiment abscised slowest of all.

To stimulate abscission most effectively, the explants must be exposed continuously to the gas. As shown in Figure 2, cotton explants exposed to ethylene for 21 hours after a 14-hour aeration period abscised more rapidly (50% increase after 35 hours) than explants given a similar 21-hour exposure immediately after cutting. This may be due to the aging response (Figure 1). When the 21-hour exposure to ethylene was divided into three 7-hour periods alternating with 7 hours of aeration, only 10% of the explants abscised after 35 hours.

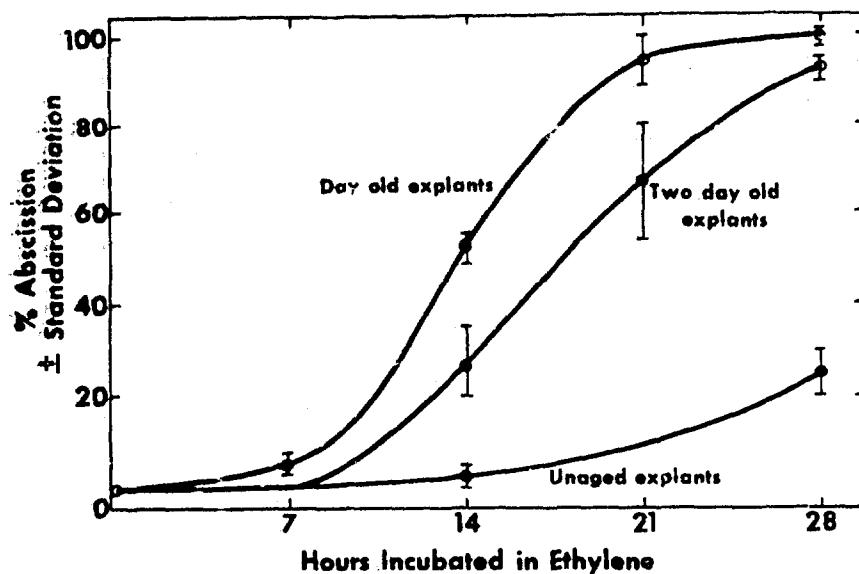


Figure 1. Effect of 0.25 nl Ethylene/ml Gas Phase on Abscission of Cotton Explants of Different Ages. Unaged explants are freshly prepared. Explants that were 24- and 48-hours old were prepared by storing for 1 or 2 days on plain agar in bottles covered with cheese cloth. Aerated control explants did not abscise during the course of this experiment. Vertical lines represent standard deviation.

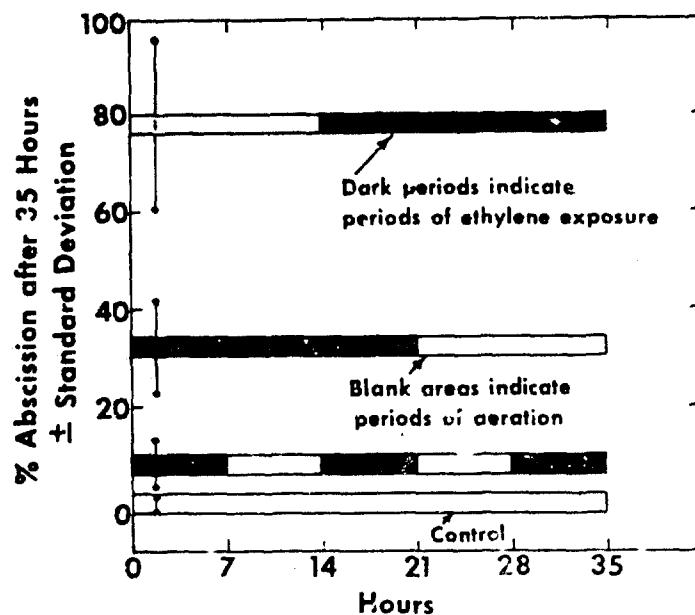


Figure 2. Effect of 21 Hours at 0.25 nl Ethylene/ml Gas Phase on Cotton Explant Abscission. Treatments started immediately after explants were harvested. Aeration was achieved by replacing rubber stopper with four layers of cheese cloth.

Experiments were then designed to measure the effects of abscission stimulators on ethylene production. 2,4-Dichlorophenoxyacetic acid and its various analogs have been reported to stimulate abscission in direct proportion to their properties as growth regulators.<sup>8,1</sup> Table 3 shows that compounds most effective in promoting ethylene evolution from cotton explants are also those most effective in promoting abscission. Although concentrations of  $10^{-4}$  M 2,5-D and 2,4-D were three to five times more effective in promoting ethylene evolution than  $10^{-5}$  M concentration of these compounds, they were only partially effective in promoting abscission, and the explants appeared flaccid and discolored. The data are expressed after the 24-hour measurement only; results were similar after 48 hours, but because of the normal course of abscission in the controls, the differences became less marked.

TABLE 3. EFFECTS OF PHENOXYACETIC ACIDS ON STIMULATION OF ETHYLENE PRODUCTION AND ABSCISSION IN COTTON

Compound	Molar Concentration	nl Ethylene/ml Gas Phase after 24 hr	% Abscission after 24 hr
Control		0.5	0
2,4-T	$10^{-4}$	0.5	0
2,6-D	$10^{-4}$	0.6	10
4-Cl	$10^{-4}$	1.0	25
2-Cl	$10^{-4}$	1.6	25
2,4,5-TISB	$10^{-4}$	2.3	75
2,5-D	$10^{-5}$	7.9	85
2,4-D	$10^{-5}$	1.9	90
		5.0	90

The stimulation of abscission and ethylene production by D- and L-amino acids is shown in Table 4. Our results (similar to those of Valdovinos and Muir)<sup>5</sup> indicate that the D-forms were more active abscission accelerators than L-forms, and our measurements show a similar capacity for the stimulation of ethylene production.

TABLE 4. RELATIONSHIP BETWEEN AMINO ACID-INDUCED<sup>a/</sup> STIMULATIONS OF ETHYLENE PRODUCTION AND ABSCISSION IN COTTON

Compound	nl Ethylene/ml Evolved		% Abscission at 48 hr
	0-24 hr	24-48 hr	
Control	0.4	0.1	0
L-glutamic acid	0.4	0.35	47
D-glutamic acid	0.6	1.6	97
L-alanine	0.5	0.25	52
D-alanine	0.7	1.8	85
L-methionine	0.8	1.0	40
D-methionine	1.2	0.8	65

a. Amino acids applied to petiole stumps as a 5- $\mu$ l drop of  $5 \times 10^{-2}$  M solution in 1% agar.

The effects of other stimulators of abscission are summarized in Table 5. It is apparent that endothal, KI and GA simultaneously increased ethylene evolution as well as the rate of abscission regardless of explant type or position of application (compare column headed "sealed" with column headed "nl C<sub>2</sub>H<sub>4</sub>/ml gas phase"). Experiments with dormin were limited to stump application to cotton explants. NAA had similar effects, but only at certain concentrations and application sites; these positional effects of auxin are discussed later. The concentrations of compounds shown in Table 5 were the most effective in stimulating abscission. Higher concentrations often gave rise to secondary effects such as flaccid and discolored tissue; more dilute concentrations had less effect on ethylene production as well as on abscission. All experiments were repeated on three separate occasions with essentially similar results.

The effectiveness of abscission-stimulating compounds was reduced by removing the accumulated ethylene from around the explants. Results are shown in Table 5. In these experiments, explants were placed in bottles covered by cheesecloth to permit equilibration of evolved ethylene with the surrounding atmosphere (column headed "aerated"). Similar bottles were capped with rubber vacine stoppers, then opened and resealed 6 and 24 hours after the start of the experiment (column headed "sealed"). It is apparent that the aeration treatment slowed rates of abscission compared with that of explants in sealed containers. Similar data were also obtained for 4 plants from coleus nodes 4 and 5. Comparable results are thus observed with each of the three different species, both sites of application, and all abscission stimulants tested. The concentrations shown in Table 5 produced moderate amounts of ethylene. Higher concentrations produced correspondingly more ethylene, but the aeration was less effective at dissipating the gas, so that much smaller differences were recorded between sealed and aerated treatments.

TABLE 5. ABSCISSION OF EXPLANTS IN SEALED AND VENTED BOTTLES

Species	Treatment	Application Site	Time of Measurement, hr	% Abscission at Time of Measurement/	
				nl C <sub>2</sub> H <sub>4</sub> /ml <sup>a</sup> / Gas Phase	Sealed Aerated
<i>Cassia</i>	Control	Bottom	48	0.05	62 35
	5 x 10 <sup>-6</sup> M NAA	Bottom	48	1.35	100 15
	10 <sup>-5</sup> M Endothal	Bottom	48	0.45	100 85
	10 <sup>-3</sup> M KI	Bottom	48	3.3	100 90
	Control	Stump	72	0.1	85 70
	10 <sup>-3</sup> M Endothal	Stump	48	0.45	100 85
<i>Coleus</i> (node 3)	Control	Bottom	48	3.6	10 5
	10 <sup>-4</sup> M NAA	Bottom	48	11.0	100 20
	10 <sup>-4</sup> M GA	Bottom	48	2.4	100 90
<i>Gossypium</i>	Control	Bottom	24	0.15	4.5 0
	10 <sup>-6</sup> M NAA	Bottom	24	2.31	100 60
	10 <sup>-5</sup> M Endothal	Bottom	24	2.01	80 5
	10 <sup>-3</sup> M KI	Bottom	24	0.24	75 0
	10 <sup>-4</sup> M GA	Bottom	48	1.44	100 0
	Control	Stump	48	0.18	70 20
	10 <sup>-1</sup> M KI	Stump	48	0.36	100 55
	10 <sup>-4</sup> M GA	Stump	48	1.37	95 75
	5 x 10 <sup>-4</sup> Dormin	Stump	48	0.51	96 75

a. Total amount of ethylene accumulated at time of measurement.

b. Bottles indicated "sealed" were opened and resealed 6 hours from the start of the experiment. The bottles were then examined vented, and resealed every 24 hours from the start until the time indicated. Bottles marked "aerated" were covered with four layers of cheesecloth in place of vaccine caps.

Carbon dioxide evolution and oxygen consumption by respiring tissues inside sealed gas collection bottles are potentially complicating factors in these experiments. In a 24-hour period representative carbon dioxide levels around explant tissue of cassia, cotton, and coleus (node 4) were 0.05, 1.1, and 2.1% v/v, respectively. The reduction in oxygen levels closely matched the increased carbon dioxide levels. Our results (similar to those of Yamaguchi<sup>22</sup>) indicated that carbon dioxide inhibits abscission. For example, 10% carbon dioxide completely blocked abscission of cotton and coleus explants and correspondingly lower levels had correspondingly less effect. However, addition of one nl ethylene per ml gas phase completely overrode the carbon dioxide inhibition. A drop in oxygen levels similar to those observed in these experiments had little effect on abscission. Abscission does not occur in the absence of oxygen.

Thus, enclosing explants in sealed bottles caused an elevation of the CO<sub>2</sub> levels which would tend to inhibit abscission. This would subsequently minimize, not magnify, any differences in abscission rates between sealed and aerated treatments.

The results obtained with auxin threaten the validity of any concept linking ethylene production to abscission. The same concentration of IAA, for example, may stimulate abscission if applied proximally, and inhibit abscission when applied distally.<sup>1</sup> However, ethylene evolution would remain the same in both instances. The following experiments in which both auxin concentration and explant length are varied show that anomalous results obtained with auxin are primarily due to diffusion phenomena.

Coleus explants were cut lengthwise into halves, each containing a petiole stump and abscission zone. These split explants were placed cut surface down on agar containing different concentrations of IAA. As shown in Figure 3, 10<sup>-3</sup> M IAA inhibited abscission as compared with that of untreated controls. Intermediate concentrations stimulated the rate of abscission, while ethylene evolution rose proportionately with increasing IAA concentrations. Essentially similar results were obtained with cotton cotyledonary node explants.

The transport path for auxin in coleus explants was also shortened by removing the stem tissue from the crotch between the two petiole stumps. Figure 4 presents data similar to that of the preceding figure. IAA could either inhibit or stimulate abscission, depending on the concentration, and ethylene evolution was directly related to the concentration of the auxin. These results for node 3 explants of coleus were repeated with explants from nodes 4 and 5 and also with cotton cotyledonary explants.

Still another method of demonstrating that the stimulatory effects of proximal auxin treatments were due to diffusion phenomena was by application of a fixed concentration of auxin to the bottom of explants whose hypocotyl tissue was cut to varying lengths. If diffusion does play a

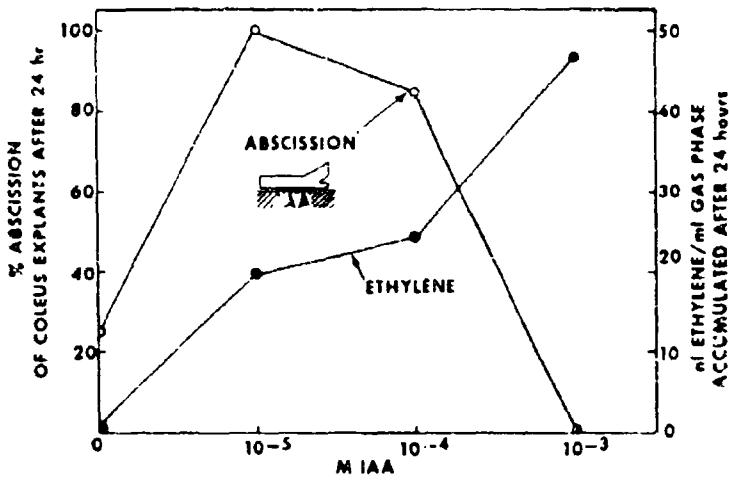


Figure 3. Inhibition and Stimulation of Abscission by Proximal Application of IAA to Split Callus Explants from Node 5.

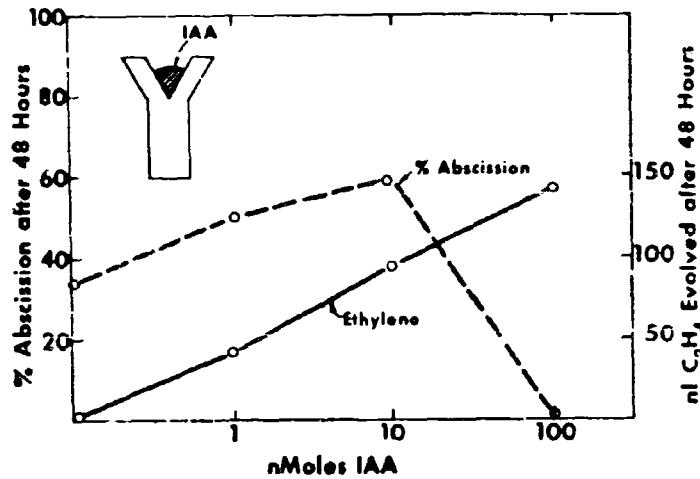


Figure 4. Effect on Abscission Activity and Ethylene Evolution of Auxin that was Applied in a 5  $\mu$ l Drop of 1% Agar to the Crotch Formed by Removing the Stem Tissue Between the Petiole Bases of Node 3.

role in accounting for the stimulatory or inhibitory effects of auxin, then NAA applied to the end of long hypocotyls should stimulate abscission (presumably due in part to ethylene production), and NAA applied to short hypocotyls should inhibit abscission (the diffusion path would be short enough for an auxin effect to take precedence). Figure 5 demonstrates that the abscission-hastening effects of  $5 \times 10^{-6}$  M and  $5 \times 10^{-4}$  M NAA are lost with a decrease in hypocotyl length, and at shorter than 8 mm lengths abscission becomes inhibited over that of the controls.

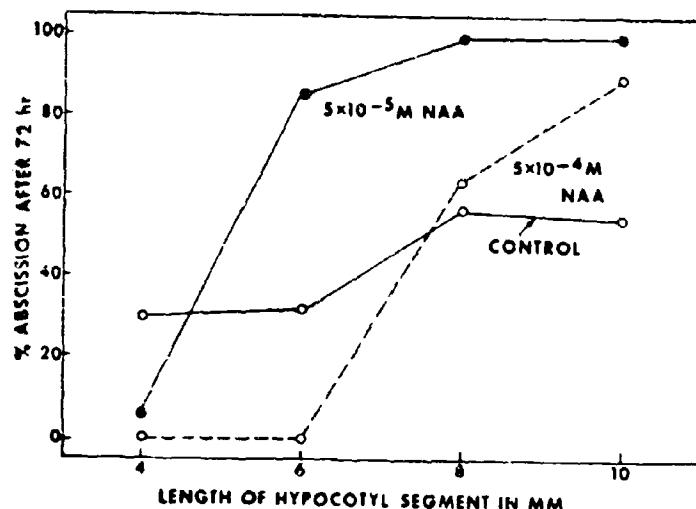


Figure 5. Effect of Decreasing the Hypocotyl Length of Cotton Explants in  $5 \times 10^{-6}$  or  $5 \times 10^{-4}$  M NAA on Abscission Rates.

#### IV. DISCUSSION

To our knowledge, all vegetative tissues evolve ethylene.<sup>18,23</sup> Equally well-documented is the ethylene-induced acceleration of abscission of leaves from intact plants and of abscission-zone explants. The extreme sensitivity of abscission zones to ethylene is illustrated in Table 2; additions of 0.1 nl/ml to the gas phase around an explant markedly accelerated its rate of abscission. On the other hand, continuous removal of endogenously produced ethylene somewhat delayed abscission compared with that of the nonaerated controls.

It seems possible, then, that the striking increases in ethylene evolution caused by auxins, endothal, KI, gibberellic acid, and amino acids from explants of bean,<sup>19</sup> cassia, cotton, and coleus may in some way participate intimately in the abscission stimulations that follow. Support for this proposal can be seen in Table 5. If the high levels of ethylene induced by the stimulatory substances are constantly removed from the atmosphere surrounding the explant, the corresponding acceleration of abscission is somewhat reduced. This response to aeration was seen for all compounds tested and for all species of explants.

Figures 1 and 2 show that the effectiveness of a given concentration of ethylene depends both on the duration of exposure and on the physiological age of the explant. This interrelationship between ethylene and the age of leaves has been described earlier. Doubt,<sup>24</sup> for example, stated that the youngest leaves were the last to abscise during prolonged treatments with illuminating gas. Later, Zimmerman et al.,<sup>3</sup> noted that the first leaves to abscise after ethylene treatments were always the oldest ones. They also showed that removing rose plants from an ethylene atmosphere (approximately 40 nl/ml) after 24 hours resulted in no acceleration of abscission; a minimum exposure of 48 hours was necessary for abscission stimulations. This suggests that, as in Figure 1, certain changes must have occurred in the leaves in the 24- to 48-hour period that precipitated that abscission response. Results of experiments in which ethylene was added at various times during development of the bean abscission zone suggested that ethylene became an effective stimulator of abscission only after the explants had been allowed to age for 48 hours.<sup>19,22</sup>

Zimmerman et al.<sup>3</sup> also noted a sharp reduction in ethylene-induced abscission of rose leaves when temperatures were lowered to 5 C. Similar results with temperature drops were mentioned by Hall and Morgan<sup>25</sup> for cotton, suggesting that the changes necessary for the abscission response to ethylene were inhibited by the colder temperatures. Data show that auxin inhibits these aging processes in bean.<sup>18,26</sup>

The nature of the changes that lead to increasing effectiveness of abscission stimulators is not known though they are associated with senescence of leaf tissue. IAA<sup>32</sup> and kinetin<sup>33</sup> which retard senescence also retard abscission. Hall and Morgan<sup>34</sup> found that high amounts of ethylene increased in vitro IAA oxidase levels in cotton plants. These observations were used to suggest that similar reactions occurred in intact plants treated with ethylene. Galston and Hillman,<sup>35</sup> however, found little evidence for the operation of such an enzyme in vivo. Burg and Burg<sup>36</sup> have shown that ethylene had no effect on auxin transport or recovery through pea-stem sections, implying that oxidase systems are not functional in intact tissue because no differences were observed in the amount of C<sup>14</sup> IAA collected at the base of ethylene-treated and control sections. Similar results using IAA-2-C<sup>14</sup> were observed with Zea mays L. coleoptiles, hypocotyls from Helianthus annus L. and Phaseolus vulgaris L., and petioles from Gossypium hirsutum L., Phaseolus vulgaris L., and Coleus blumei Benth.<sup>31</sup> Further, GA that stimulated both ethylene production and abscission (Table 3) has been reported to inhibit IAA oxidase action in vitro.<sup>32,33</sup> Thus it does not appear that changes leading to abscission are caused by ethylene acting directly on auxin metabolism.

If ethylene did stimulate in vivo processes leading to aging and subsequently the response to stimulatory substances, it would be difficult to explain why short exposures to the gas fail to increase abscission over untreated controls<sup>3,18,32</sup> (Figures 1 and 2). Although it is impossible at this time to characterize further the nature of the increase in sensitivity to ethylene, the metabolism of the plant as it ages must be taken into consideration when studying the control of leaf abscission.

Objections that internally produced ethylene might regulate leaf fall were proposed by Addicott and Lynch<sup>34</sup> and Jacobs.<sup>35</sup> Addicott's group cite experiments in which air was analyzed over a cotton field containing abscising flowers and detectable amounts of ethylene were not found. Results by Hall et al.,<sup>36</sup> however, have shown quite clearly that cotton plants of all ages do produce measurable quantities of ethylene, suggesting that the data cited by Addicott and Lynch resulted from not sampling close enough to the plant. This criticism points out some of the difficulties encountered when analyzing interactions of ethylene with vegetative tissues. One must assume that ethylene is produced endogenously by the vegetative tissues and that the gas also acts within the tissues. Thus, the measured ethylene could be considered as the amount that is not used by the plant.

Jacobs<sup>35</sup> found that an intact coleus leaf stimulated abscission of the debladed petiole directly below it. When no abscission stimulation was obtained by placing a plant with debladed petioles in intimate contact with intact leaves from another plant, he concluded that the leaf above the debladed petiole was not the cause for the observed stimulation. However, it is possible that a substance, presumably an auxin,<sup>37</sup> moving down from that leaf might promote petiolar abscission below by the subsequent production of ethylene.

It is also not likely that ethylene is merely a byproduct of abscission. If this were so, its addition to explants would induce further production of the gas by the tissue. Such an autocatalytic effect was never observed.

The auxin-gradient theory of Addicott and Lynch<sup>1</sup> appears to contradict the proposal that ethylene produced by auxin participates in abscission. Addicott and Lynch interpreted their data as showing that auxin control of abscission is dependent primarily on the site of application and not on concentration or time of application. Since auxin is transported in petioles in a strongly polar manner,<sup>38</sup> it is possible that the positional effects<sup>34</sup> are due to the presence of different concentrations of auxin near the abscission zone. Thus, distally applied auxin would be transported rapidly in a polar manner and could prevent any aging processes leading to abscission. Auxin applied proximally, however, must move acropetally, primarily by diffusion, resulting in less hormone accumulating later at the abscission zone than if it were applied distally.

We attempted to check this supposition by altering the rate of diffusion of auxin to the abscission zone either by raising the concentration of auxin or by leaving the concentration constant and decreasing the diffusion path. Figures 3 and 4 show that the proximally induced stimulation of abscission predicted by the auxin-gradient theory can be reversed by increasing the concentration of auxin. Similar results with bean explants have been reported earlier by Gaur and Leopold.<sup>35</sup> In an experiment by Kaushik<sup>40</sup> (his Figure 11) which was similar to that shown in Figure 3, a stimulation of abscission with low concentrations of IAA was not observed. However, a strict comparison between Kaushik's and our experiments is not possible, as his explants were incubated in the dark and ours in the light. Speeding diffusion by shortening the diffusion path also led to abscission inhibitions, as shown in Figure 5. In this case, the same concentration of proximally applied auxin that accelerated abscission of 10-mm hypocotyls inhibited abscission of 4-mm hypocotyls. If we then assume that the positional effects of auxin are due only to different concentrations appearing at the abscission zone at different times, the results of Addicott and Lynch may be explained with the aid of auxin-induced ethylene production. Thus, high concentrations of auxin in the abscission zone, as might appear soon after applications of high concentrations and/or distal applications, would result in an inhibition of aging processes<sup>28</sup> that normally lead to a sensitivity to ethylene followed by abscission.<sup>18</sup> More dilute concentrations of auxin at the abscission zone caused by the addition of less auxin and/or proximal applications would induce the formation of ethylene, as did higher concentrations, but could not retain the physiologically youthful condition during which abscission is resistant to the gas. In this case an acceleration of abscission would result.

Hypotheses considering the essentiality of ethylene in leaf abscission have centered around a balance between growth regulators and the amount of gas being produced by the leaf. Gawadi and Avery<sup>41</sup> suggested a "hormone-ethylene" theory in which the ethylene was thought to stimulate the process of aging. Hall<sup>42</sup> stated that abscission can occur whenever IAA synthesis is reduced or ethylene evolution increased.

Results of altering the auxin levels also provide evidence against a strict control of abscission by a hormone-ethylene balance. It can be shown that dilute concentrations of auxin,<sup>39,43</sup> or auxin applications to aged (18-hour) explants,<sup>38</sup> can stimulate abscission. If a drop in auxin level is a prerequisite to abscission, then a stimulation of abscission by auxin is opposite to what is expected by the earlier theories. Similarly, the marked increase in ethylene evolution during auxin treatments that inhibit abscission<sup>17,18</sup> speaks against the balance theory.

Our view hinges on the consideration of leaf abscission as a natural consequence of the processes of senescence in the foliar tissue. As the leaf ages, various metabolic alterations occur, so that the aged leaf is quite different physiologically from young, vigorous tissue. A high auxin level appears to be able to prevent<sup>27</sup> the onset of senescent changes, although not to reverse them after they have occurred. Ethylene, meanwhile, is an active stimulator of abscission only when applied to older tissues. Therefore, it is not a matter of a promotion of ethylene or a decrease of auxin that basically determines abscission rates, but is instead an increase in sensitivity of the tissue to the ethylene that is already being produced. An essentially similar hypothesis has been advanced earlier by Barlow.<sup>44,45</sup>

Our statements concerning the importance of ethylene for the induction of leaf abscission should not be construed as meaning that this is the sole compound involved. Although we have never found a compound (or environmental condition) that accelerated abscission without concurrently stimulating ethylene evolution, it cannot be assumed that such a compound (or condition) does not exist. Further, it must be remembered that certain substances may have other effects on abscission besides stimulating ethylene. As examples, Bormann<sup>46</sup> has shown that GA can induce abscission-zone formation in cotton stems, and Mitchell et al.<sup>47</sup> have demonstrated a similar phenomenon in bean stems using naphthalylphthalamic acid.

Although there is a circumstantial relationship between ethylene production and leaf abscission, there is no evidence for an absolute requirement for this gas in order for abscission to occur. It is impossible to establish this because small quantities of ethylene are always present in vegetative tissue. What is known is that ethylene accelerates abscission processes once the appropriate stage of senescence has been reached, and any treatment that accelerates ethylene evolution without interfering with the natural aging of the cell will accelerate abscission. Using this interpretation, it is possible to unify a great deal of the literature pertaining to the effects of various compounds on abscission.

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13. ABSTRACT  Abscission zone explants of <u>Gossypium hirsutum</u> L., <u>Cassia fistula</u> L., and <u>Coleus blumei</u> Benth. were used to investigate correlations between endogenous rates of ethylene evolution and time of abscission. Additions of 0.1 nanoliter per milliliter ethylene to the explants markedly accelerated abscission; continuous aeration of the explants, to prevent accumulation of small amounts of endogenously produced ethylene, inhibited abscission compared with that of sealed controls. Substances that stimulated abscission simultaneously accelerated ethylene evolution on all three species and at any position of application.		
 The positional effects of auxin are explained as being due to differences in transport in the explant. Thus, distally applied auxin inhibits abscission regardless of the accelerated rate of ethylene evolution by being rapidly transported to the abscission zone. Auxin applied proximally stimulates abscission because it is unable to move as rapidly to the abscission zone and the ethylene effect becomes dominant.		
 Ethylene was found to be most effective at longer exposures and on aged tissues, and it is concluded that abscission rates are not determined basically by an auxin-ethylene balance but by an increase in sensitivity of the tissue to the ethylene that is already being produced.		

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